Published in final edited form as:
Clin Transplant. 2010 ; 24(3): E54–E61. doi:10.1111/j.1399-0012.2010.01243.x.

A review of the potential applications and controversies of non-invasive testing for biomarkers of aspiration in the lung transplant population

C.S. Davis[a], **J. Gagermeier**[b], **D. Dilling**[b], **C. Alex**[b], **E. Lowery**[c], **E.J. Kovacs**[d], **R.B. Love**[c], and **P.M. Fisichella**[a]

[a]Department of Surgery, Loyola University Medical Center, Maywood, IL, USA

[b]Division of Pulmonary and Critical Care Medicine, Loyola University Medical Center, Maywood, IL, USA

[c]Division of Cardiothoracic Surgery, Loyola University Medical Center, Maywood, IL, USA

[d]Burn and Shock Trauma Institute, Loyola University Medical Center, Maywood, IL, USA

Abstract

Despite improvements in one-yr survival following lung transplantation, five-yr survival lags significantly behind the transplantation of other solid organs. The contrast in survival persists despite advancements in anti-rejection regimens, suggesting a non-alloimmune mechanism to chronic lung transplant failure. Notably, markers of aspiration have been demonstrated in bronchoalveolar lavage (BAL) fluid concurrent with bronchiolitis obliterans syndrome (BOS). This recent evidence has underscored gastro-esophageal reflux (GER) and its associated aspiration risk as a non-alloimmune mechanism of chronic lung transplant failure. Given the suggested safety and efficacy of laparoscopic anti-reflux procedures in the lung transplant population, identifying those at risk for aspiration is of prime importance, especially concerning the potential for long-term improvements in morbidity and mortality. Conventional diagnostic methods for GER and aspiration, such as pH monitoring and detecting pepsin and bile salts in BAL fluid, have gaps in their effectiveness. Therefore, we review the applications and controversies of a non-invasive method of defining reflux injury in the lung transplant population: the detection of biomarkers of aspiration in the exhaled breath condensate. Only by means of assay standardization and directed collaboration may such a non-invasive method be a realization in lung transplantation.

Keywords

aspiration; bronchiolitis obliterans syndrome; chronic allograft dysfunction; exhaled breath condensate; gastroesophageal reflux; lung transplantation

Brief review of lung transplantation

Lung transplantation has now become acceptable palliation for the end-stage consequences of many pulmonary diseases (1). From January 1995 to June 2007, the most common indications for lung transplantation included chronic obstructive pulmonary disease (COPD,

Corresponding author: Piero M. Fisichella, MD, Swallowing Center, Department of Surgery, Stritch School of Medicine, Loyola University Medical Center, 2160 South First Avenue – Room 3226, Maywood, IL 60153, USA. Tel.: +708 327 2820; fax: +708 327 3492; pfisichella@lumc.edu.

36%), idiopathic pulmonary fibrosis (IPF, 20%), cystic fibrosis (CF, 16%), and α_1-anti-trypsin deficiency emphysema (AAT, 8%) (2). Improvements in surgical technique and enhanced medical therapies have afforded lung transplant recipients improved survival, especially at the one-yr mark (2,3). The one-yr survival rate has increased from 74% to 81% and the five-yr survival rate from 47% to 54% (2). Unfortunately, according to the International Society for Heart and Lung Transplantation (ISHLT) Twenty-fifth official report, the long-term survival among one-yr survivors remains unchanged (6.9, 7.2, and 7.1 yr across eras starting in 1988) (2). Moreover, five-yr survival continues to hover around a dismal 50%, lagging significantly behind other solid-organ transplantation such as liver, kidney, and heart, which have demonstrated recipient and graft five-yr survival of over 70% (4–6). Thus, despite advancements in abilities to mitigate common causes of death within the first 12 months post-transplant, our common understanding and modes of therapy are falling short of the ultimate goal, which is to offer patients long-term quality and quantity of life.

Chronic allograft rejection

Chronic rejection has been dubbed the “Achilles heel” of long-term lung transplant success (7). Chronic transplant deterioration occurs as a consequence of fibrous obliteration of the small airways. This is known as bronchiolitis obliterans (OB), whereby fibrous obliteration is the result of macrophage and myofibroblast infiltration and fibroproliferation (8,9). Given that OB is a histologic diagnosis mandating the invasiveness of surgical biopsy, the clinical correlate bronchiolitis obliterans syndrome (BOS) is often applied. This was originally defined as a persistent drop in forced expiratory volume in one s (FEV_1) by 20% in the absence of other identifiable causes (10). The significance of BOS in predicting poor long-term outcome subsequently led to the adjustment of criteria to include an early BOS stage (BOS 0-p) in which there exists an FEV_1 of 81–90% and/or a drop in midexpiratory flow rate ($FEF_{25–75}$) (11). Indeed, the prevalence of BOS following lung transplant and its ability to predict unacceptable outcomes are striking. The ISHLT notes that among more than 10 000 recipients living longer than 14 d, there is an occurrence of 27% with BOS by 2.5 yr post-transplant, and 51% by 5.6 yr (2). Furthermore, survival at five yr is 20–40% lower for those with BOS than without. After the onset of BOS, only 30–40% survive an additional five yr (12). Therefore, BOS is a progressive and unrelenting process with limited effectiveness in treatment strategies.

The imperative is thus to understand the relationship of BOS to its risk factors such that appropriate methods may be employed to predict future occurrence (13). Theories for the underlying mechanism of OB/BOS are abundant and the process is undoubtedly multifactorial. Risk factors appear to be alloimmune and non-alloimmune, hinging on a dysfunctional response by innate and adaptive mechanisms of immunity (14). Severe and repeated acute rejection episodes, HLA mismatching, and anti-HLA antibodies are possible alloimmune links to OB/BOS (14). Conversely, non-alloimmune culprits in the histopathologic genesis of OB seem to include infection, ischemia, and as we wish to address, gastroesophageal reflux (GER) (11,12,14).

From GER to aspiration

The commonality between GER and respiratory diseases, such as asthma, cystic fibrosis, and IPF, has been well studied over the course of decades (15–19). Likewise, the increased prevalence of GER in the lung transplant population has been recently characterized. Hadjialidis et al. (20), by esophageal pH monitoring, demonstrated a post-transplant prevalence of distal esophophageal reflux of 69.8%. Similarly, Young et al. showed an increased prevalence of distal esophageal reflux from 34.8% to 65.2% following lung

transplantation; D'Ovidio et al. had nearly equivalent findings in the rise of reflux prevalence post-transplant from 32% at three months to 53% at 12 months (21,22). Furthermore, by means of heightening the likelihood of aspiration, the significance of the increased prevalence of GER after lung transplantation cannot be overstated, especially given implications in the pathogenesis of OB/BOS (23–26). Abernathy et al. (27) described two histologic forms of OB in lung transplant patients, one being an acellular concentric fibrosing process in the terminal bronchioles, and the other a focal cellular process extending to the distal alveolar spaces associated with foreign body-type giant cells and aspirated material. Concurrent with the above, an animal model of chronic aspiration has recently demonstrated accelerated allograft failure and histologic findings similar to OB (28,29). Although relevant and not to be discredited, the studies do not unfortunately address which component(s) of gastric aspiration may be contributory, nor do they mimic the unproven theory that the pathodevelopment of BOS in humans is at least in part related to chronic microaspiration and not solely macroaspiration events (30–32).

The increased risk for reflux and aspiration post-lung transplantation appears to be a function of delayed gastric emptying, esophageal dysmotility, and/or a hypotensive lower esophageal sphincter (LES), all of which are potentially related to medications (especially immunomodulating drugs) or physical alterations secondary to chronic lung disease or iatrogenic vagal nerve injury (22,26,33–35). This enhanced risk for reflux and aspiration, in combination with altered mucociliary clearance and cough, leaves the transplanted lung vulnerable to injury. The imperative for determining those at risk for non-alloimmune BOS relies upon the availability of anti-reflux surgery, with the endpoint of protecting the transplanted lung from reflux and aspiration-related damage.

Anti-reflux options

GER has been recognized for some time as a complicating factor in lung transplantation. Given its ties to the promotion and/or acceleration of BOS, the importance of managing reflux has come forth. Medical modes of therapy rely on conventional methods that typically consist of histamine blockers, proton-pump inhibitors (PPIs), and pro-motility agents. Though these agents may ameliorate symptoms, diminish the acid component of gastric refluxate, and improve gastroparesis, the underlying mechanical mechanism for reflux often persists (36,37). If so, and in the setting of diagnosed pathologic reflux, the lung transplant patient may be offered fundoplication, of which the safety and efficacy in this special population is in the process of delineation (23–26,38).

Even in the face of inherent comorbidities and immunosuppressive regimens related to end-stage lung disease and lung transplantation, the growing body of evidence for the utility of anti-reflux surgery in these patients is intriguing (38–42). There appears to be an association to fundoplication delaying the onset of BOS, if not even improving lung allograft function when BOS is present (40,43,44). Cantu et al. demonstrated no evidence of BOS at one and three yr when anti-reflux surgery was performed within one month of lung allograft. Comparatively, 96% of lung recipients without anti-reflux surgery had BOS at one yr, and 60% at three yr (45). Further evidence for the application of anti-reflux surgery in the transplant population was demonstrated by Lau et al. (39), who reported that 67% of lung transplant recipients had improvement in their pulmonary function following surgical anti-reflux control. Likewise, Davis et al. (44) had similar findings given that 81% of patients in their study no longer met the criteria for BOS after anti-reflux surgery. This latter group also concluded that actuarial survival was significantly better in those without GER or who had undergone an anti-reflux procedure (44). Yet, to further substantiate these promising findings, objective studies are required to better understand the safety and efficacy of anti-reflux surgery in lung transplantation.

In their study on laparoscopic Nissen fundoplication in lung transplant recipients, O'Halloran et al. (42) included a control group for comparison. Here, the lung transplant group did have a longer hospital length of stay (2.89 versus 0.71 d) and a higher rate of readmission (25% versus 3%) when compared to controls. This was attributed to higher operative risk and pulmonary as opposed to gastrointestinal status. Nonetheless, operative time and estimated blood loss did not reach statistical significance. All told, there were minimal complications, no intraoperative or perioperative deaths, and both groups had similar resolution of their reflux symptoms. In contrast to this study, our institution's findings indicate an even more favorable safety profile. When compared to controls, lung transplant patients had similar complication rates ($p = 0.88$) and hospital length of stay (1.7 versus 1.8 d; $p = 1.0$), with no in-hospital or 30-d mortality (abstract in submission). That said, some facets continue to require objective determination, such as the timing of intervention and the effects on the natural history of BOS as they relate to anti-reflux surgery in the lung transplant patient.

In summary, evidence is mounting to suggest reliable safety and efficacy of LARS in lung transplantation. As GER is increasingly implicated in long-term allograft function, the onus is to determine those at greater risk for reflux such that medical and surgical therapies might be implemented early, possibly even before transplant itself (38,40,41). The issue in this process is screening.

Screening for reflux

Given the prevalence of GER in advanced lung disease (19,46), and in the lung transplant population itself (20,22,45,47), it is important to predict those at risk for aspiration, as well as to discern which components of reflux and aspiration may be most contributory to the development of BOS. In this way, anti-reflux methods may be deployed prior to irreversible change.

Traditional gastrointestinal symptoms, such as heartburn and regurgitation, are an unreliable correlate between reflux and airway disease (31,48–50). Simple reliance on a high index of suspicion in those with atypical reflux is difficult and unacceptable (51). Additional investigation with esophageal pH monitoring, though considered the gold standard for confirming GER, is presumptuous in those with associated airway disease (49,52). Indeed, Oelschlager et al. (49) in 2005, in a study population of 518 consecutive patients, found even the combination of symptoms, esophageal manometry, and standard esophageal pH monitoring to be insufficient in accurately identifying reflux as the cause of aspiration. Positive esophageal pH testing does necessarily mean that reflux is the cause of airway disease, nor does negative esophageal pH testing rule-out aspiration (49). Further, esophageal pH testing does not address the issue that abnormal pulmonary symptoms could both be the result or cause of GER (53). Esophageal pH monitoring, when combined with pharyngeal pH monitoring, has also been posited as a predictor of response to both medical and surgical management (52,54). Unfortunately, ambulatory 24-h dualchannel pH monitoring has several limitations. Its sensitivity for reflux is only 50% to 80% (50,55), it is not always well tolerated (50), and it is both expensive and invasive. As such, a true gold standard is lacking in the assessment of reflux and aspiration, in that those with negative pharyngeal pH monitoring may nonetheless have aspiration-related airway disease (49). Further still, esophageal and pharyngeal pH monitoring do not distinguish acid from non-acid GER. Therefore, alternative means must be considered.

Esophageal multichannel intraluminal impedance offers the unique advantage of quantifying GER irrespective of acid content (56). Tamhankar et al. (36) demonstrated that PPIs do not reduce frequency of reflux episodes, solely altering the refluxate from acidic to non-acidic.

A more recent study of lung transplant patients characterized acid and non-acid reflux as measured by esophageal impedence with markers of aspiration detected by bronchoalveolar lavage (BAL). In this study, 71% of lung transplant recipients taking PPIs had increased non-acid GER, and overall PPIs did not reduce the total number of reflux events, the number of non-acid reflux events, volume exposure, proximal extent of reflux, or markers of aspiration in BAL (37). These findings are important given the suggestion that non-acid GER, not detected by conventional esophageal monitoring, is also a risk factor for aspiration of gastroduodenal contents. Indeed, Blondeau et al. (37) found no difference in pepsin or bile acid detection by BAL in transplant patients treated, or not treated, with PPIs. This indicates that perhaps surgical control of reflux is the only recourse for true prevention of aspiration, raising the importance for selecting patients that would benefit from fundoplication. Unfortunately, esophageal impedance testing, much like esophageal and pharyngeal pH monitoring, is only a surrogate marker of aspiration. In that respect, recent work at detecting markers of aspiration in BAL fluid post-lung transplant has yielded promising results and a renewed focus on the role of GER in BOS.

Surveillance BAL post-lung transplant has become routine in some centers, despite controversy in its promoting a survival advantage (57,58). Nevertheless, bronchoscopy, transbronchial biopsy, and BAL do afford the ability to diagnose early acute rejection, infection, and to evaluate cells and soluble components of the alveolar lining (58–60). With regards to reflux, markers of aspiration such as bile acids and pepsin have been demonstrated in BAL fluid of post-lung transplant patients (21,24,25,37,61,62). This provides direct evidence for the aspiration of gastroduodenal contents into the transplanted organ (24,37,61). Furthermore, the authors of these studies have demonstrated the presence of bile acids and pepsin in concurrence with rejection and BOS. D'Ovidio et al. (24) found a 17% prevalence of high concentrations of bile acids, with most prominent levels of bile acids in 70% of those with early onset (defined as less than one yr post-transplant) and high severity of BOS. In another study, D'Ovidio et al. (21) showed a time and dose-dependent development of BOS when bile acids were present at three months post-lung transplant. The incidence of BOS in this study among those with high levels of bile acids was four times that of patients with undetectable levels. Along these same lines, pepsin in the BAL fluid is also common to the post-lung transplant patient. Ward et al. (61) found pepsin in the BAL fluid of all 13 lung allografts, with none in that of the control group. Likewise, Stovold et al. (62) showed in their study of 36 patients consistently elevated levels of pepsin in the BAL fluid of lung transplant patients, with the highest levels associated with acute rejection. Similarly, Blondeau et al. (37) found pepsin in the BAL fluid of all patients with reflux after lung transplant. This study contrasted that of Stovold et al. in that elevated levels of pepsin were not detected in those with BOS. However, in the 50% of lung transplant patients that demonstrated elevated levels of bile acids, 70% of those with BOS had elevated bile acids when compared to 31% without BOS. In concert with prior studies, that of Blondeau et al. suggests that pepsin might be a sensitive marker of aspiration, and bile acids a specific marker for BOS. Not only then are bile acids and pepsin common in the BAL fluid of lung-transplant patients, their presence is associated with the development of acute rejection and BOS in such a fashion as to be potentially useful as a clinical screening and diagnostic tool.

Though overall promising given its status as a burgeoning method, the detection of bile acids and pepsin in BAL fluid has specific concerns. First, early cross-sectional studies only provide a snapshot in time of biomarker presence, for which there is no standardization in detection assay, biomarker concentration, patient follow-up, or comparison to clinical, laboratory, and pathologic findings. Second, there is no information on how bile acid and pepsin concentrations change over time following aspiration, or with frequency of aspiration events. Third, alterations in content and concentrations may be dependent on BAL technique, lending the possibility for variability in results secondary to dilution, pH,

bleeding, and inflammation from instrumentation. As such, there is a void in the standardization of methods to collect and assay markers of reflux and aspiration in the lung-transplant patient. BAL has utility and an established basis for surveillance of rejection, though given its invasiveness, on rare occasions major complications do occur (60). For this reason, exhaled breath condensate (EBC) offers a unique potential in the aspiration workup, with the added benefit of safety in the lung transplant population.

Exhaled breath condensate

First described in 1980 (63), EBC was largely ignored in the international community until this current decade (64), and it is an evolving method for the non-invasive assessment of pulmonary disease (65–68). During collection, the patient comfortably breathes for approximately 10 minutes into a cooled plastic cylinder. The condensate can then be collected and assayed for numerous biomarkers of disease, notably protons (by means of pH), hydrogen peroxide, nitric oxide, and cytokines (67). Overall, much of the utility of EBC is unknown, specifically in the determination of reference ranges for specific biomarkers and protocols for their detection assays. That said, effort has been undertaken by the American Thoracic Society/European Respiratory Society (ATS/ERS) Task Force on Exhaled Breath Condensate to provide reliable guidelines for methods of collection and biomarker analysis (68). The Task Force states that EBC is “a useful tool for epidemiological investigation…to help gain understanding of the time courses of important pathologic processes” (68).

Much controversy still exists regarding EBC, especially in its comparison with BAL. Types and concentrations of biomarkers in EBC compared to BAL have been evaluated with mixed results. Ono et al. (69) did demonstrate a correlation in cysteinyl leukotriene concentration between EBC and BAL. However, in a study by Jackson et al. (70) that involved a subset of 26 lung transplant patients, no significant correlation was found between EBC and BAL for any biomarker evaluated, either before or after correction for dilution. Nonetheless, Jackson et al. (70) found that most biomarkers were found in higher concentrations in EBC than BAL and that biomarkers from EBC were easily measured with commercially available assays. These results substantiate that EBC has clinical potential in the assessment of patients with airway disease, with the caveat that the detection of biomarkers in EBC should be conceptualized differently than in BAL, especially given further unknowns along the lines of solubility, volatility, and electric charge.

With the above limitations in mind, recent work with EBC has been performed specific to the field of lung transplantation itself. Dupont et al. (71) found that a low pH in EBC correlated with both acute rejection and BOS. Furthermore, these authors demonstrated concurrence of pH levels in EBC with the incidence, severity, and progression of BOS. Intuitively, reflux and aspiration have the potential to lower pH in the distal airway. Hunt et al. (72) demonstrated temporal relationship to lowered pH of EBC following acid aspiration. Alternative explanations for airway acidification include infection and the physiology of the allograft itself, in that neutrophilic inflammation may be a source of lowered pH (73). Therefore, these findings may or may not be related to GER. Nonetheless, the association between the pH of EBC and graft dysfunction is not discounted.

Novel applications for the non-invasive utility of EBC in lung transplant patients are being elucidated, an example of which is the detection of pepsin. Krishnan et al., in an abstract, demonstrated pepsin in EBC of lung transplant patients, finding significant pepsin levels in transplant versus control EBC ($p = 0.004$). Furthermore, they found a meaningful drop of pepsin in EBC following Nissen fundoplication ($p = 0.009$). Incidentally, they also showed a significant rise in pH post-anti-reflux procedure (0.02) (74).

To date, few centers have investigated other biomarkers of reflux and aspiration in EBC, such as bile acids, rendering avenues for advancement. Thus, EBC shows promise as a reproducible, non-invasive method of detecting biomarkers of aspiration in the setting of lung transplant patients and their predisposition for reflux. However, much work must be undertaken for this promise to come to fruition. Furthermore, the search for meaningful markers of aspiration in BAL fluid must also continue, especially in light of surveillance bronchoscopy that is standard of care in many lung transplant centers. Via a multidisciplinary team consisting of clinicians and laboratory scientists, our center has begun this endeavor. Through the collection of EBC and BAL fluid, we will be afforded direct comparison of invasive and non-invasive means of detecting aspiration biomarkers. Additional effort is also underway to standardize the assays that detect these biomarkers such that future research collaboration and clinical application can be achieved.

Conclusions

Long-term survival following transplantation of the lung is significantly less than that of other solid organs, the reasons for which are not fully understood. A well-supported theory relates reflux to aspiration and the subsequent development of BOS. Undoubtedly, there is an elevated prevalence of GER in those with end-stage lung disease, and more so following lung transplantation. Given that anti-reflux surgery appears to be safe and reliable even in these comorbid patients, the quintessential directive is to determine those at risk for reflux and aspiration such that quality and quantity of life can be sustained. The addition of esophageal multichannel intraluminal impedance to standard methods of reflux detection may complete the picture in esophageal function testing by detecting non-acid components of GER. Next, BAL is nearest at assessing post-lung transplant patients for the risk of allograft injury from aspiration. However, little is known whether the analysis of BAL fluid for markers of aspiration is indeed the best method. As such, a non-invasive and reliable screening method, such as EBC, defines itself as a safe alternative for the detection of reflux biomarkers from the lung. Because EBC is still in its infancy as an application to lung transplantation, the foremost first step is to foster a standardization of collection methods and biomarker detection. Additionally, inadequate sensitivity and specificity must be addressed, a fact that holds true not just for EBC but for BAL fluid as well. Finally, the overall imperative remains: the application of the most appropriate intervention at the most appropriate time. This may be achievable through standardization of screening methods for GER and aspiration risk in the lung transplant recipient. To these ends, we favor aggressive research and directed collaboration to make such promising applications a reality for the lung transplant patient.

Acknowledgments

This work was supported by: Dr. Ralph and Marian C. Falk Medical Research Trust.

References

1. Wilkes DS, Egan TM, Reynolds HY. Lung transplantation: opportunities for research and clinical advancement. Am J Respir Crit Care Med. 2005; 172:944. [PubMed: 16020804]
2. Christie JD, Edwards LB, Aurora P, et al. Registry for the International Society for Heart and Lung Transplantation: twenty-fifth official adult lung and heart/lung transplantation report. J Heart Lung Transplant. 2008; 27:957. [PubMed: 18765187]
3. Garrity ER, Moore J, Mulligan MS, Shearon TH, Zucker MJ, Murray S. Heart and lung transplantation in the United States, 1996–2005. Am J Transplant. 2007; 7(5 Pt 2):1390. [PubMed: 17428287]

4. Brown RS, Rush SH, Rosen HR, et al. Liver and intestine transplantation. Am J Transplant. 2004; 4 Suppl. 9:81. [PubMed: 15113357]
5. Wynn JJ, Distant DA, Pirsch JD, et al. Kidney and pancreas transplantation. Am J Transplant. 2004; 4 Suppl. 9:72. [PubMed: 15113356]
6. Pierson RN 3rd, Barr ML, McCullough KP, et al. Thoracic organ transplantation. Am J Transplant. 2004; 4 Suppl. 9:93. [PubMed: 15113358]
7. Orens JB, Garrity ER Jr. General overview of lung transplantation and review of organ allocation. Proc Am Thorac Soc. 2009; 6:13. [PubMed: 19131526]
8. Yousem SA, Berry GJ, Cagle PT, et al. Revision of the 1990 working formulation for the classification of pulmonary allograft rejection: lung rejection study group. J Heart Lung Transplant. 1996; 15(1 Pt 1):1. [PubMed: 8820078]
9. Grossman EJ, Shilling RA. Bronchiolitis obliterans in lung transplantation: the good, the bad, and the future. Transl Res. 2009; 153:153. [PubMed: 19304274]
10. Cooper JD, Billingham M, Egan T, et al. A working formulation for the standardization of nomenclature and for clinical staging of chronic dysfunction in lung allografts. International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 1993; 12:713. [PubMed: 8241207]
11. Estenne M, Maurer JR, Boehler A, et al. Bronchiolitis obliterans syndrome 2001, an update of the diagnostic criteria. J Heart Lung Transplant. 2002; 21:297. [PubMed: 11897517]
12. Valentine VG, Robbins RC, Berry GJ, et al. Actuarial survival of heart-lung and bilateral sequential lung transplant recipients with obliterative bronchiolitis. J Heart Lung Transplant. 1996; 15:371. [PubMed: 8732596]
13. Estenne M, Hertz MI. Bronchiolitis obliterans after human lung transplantation. Am J Respir Crit Care Med. 2002; 166:440. [PubMed: 12186817]
14. Nicod LP. Mechanisms of airway obliteration after lung transplantation. Proc Am Thorac Soc. 2006; 3:444. [PubMed: 16799090]
15. Belcher JR. The pulmonary complications of dysphagia. Thorax. 1949; 4:44. [PubMed: 18113256]
16. Davis MV. Evolving concepts regarding hiatal hernia and gastroesophageal reflux. Ann Thorac Surg. 1969; 7:120. [PubMed: 5763516]
17. Pearson JE, Wilson RS. Diffuse pulmonary fibrosis and hiatus hernia. Thorax. 1971; 26:300. [PubMed: 5089496]
18. Mays EE, Dubois JJ, Hamilton GB. Pulmonary fibrosis associated with tracheobronchial aspiration. A study of the frequency of hiatal hernia and gastroesophageal reflux in interstitial pulmonary fibrosis of obscure etiology. Chest. 1976; 69:512. [PubMed: 1261317]
19. Tobin RW, Pope CE 2nd, Pellegrini CA, Emond MJ, Sillery J, Raghu G. Increased prevalence of gastroesophageal reflux in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1998; 158:1804. [PubMed: 9847271]
20. Hadjiliadis D, Davis RD, Steele MP, et al. Gastro-esophageal reflux disease in lung transplant recipients. Clin Transplant. 2003; 17:363. [PubMed: 12868994]
21. D'Ovidio F, Mura M, Ridsdale R, et al. The effect of reflux and bile acid aspiration on lung allograft and its surfactant and innate immunity molecules SP-A and SP-D. Am J Transplant. 2006; 6:1930. [PubMed: 16889547]
22. Young LR, Hadjiliadis D, Davis RD, Palmer SM. Lung transplantation exacerbates gastroesophageal reflux disease. Chest. 2003; 124:1689. [PubMed: 14605036]
23. D'Ovidio F, Keshavjee S. Gastroesophageal reflux and lung transplantation. Dis Esophagus. 2006; 19:315. [PubMed: 16984525]
24. D'Ovidio F, Mura M, Tsang M, et al. Bile acid aspiration and the development of bronchiolitis obliterans after lung transplantation. Thorac Cardiovasc Surg. 2005; 129:1144.
25. D'Ovidio F, Mura M, Waddell TK, et al. Bile acids in bronchoalveolar lavage after lung transplantation as a marker of pulmonary aspiration associated with alveolar neutrophilia. J Heart Lung Transplant. 2005; 23 Suppl. 1(2):42S.
26. Reid KR, McKenzie FN, Menkis AH, et al. Importance of chronic aspiration in recipients of heart-lung transplants. Lancet. 1990; 336:206. [PubMed: 1973771]

27. Abernathy EC, Hruben RH, Baumgartner WA, Reitz BA, Hutchins GM. The two forms of bronchiolitis obliterans in heart-lung transplant recipients. Hum Pathol. 1991; 22:1102. [PubMed: 1743693]
28. Hartwig MG, Appel JZ, Li B, et al. Chronic aspiration of gastric fluid accelerates pulmonary allograft dysfunction in a rat model of lung transplantation. J Thorac Cardiovasc Surg. 2006; 131:209. [PubMed: 16399314]
29. Li B, Hartwig MG, Appel JZ, et al. Chronic aspiration of gastric fluid induces the development of obliterative bronchiolitis in rat lung transplants. Am J Transplant. 2008; 8:1614. [PubMed: 18557728]
30. Sweet MP, Patti MG, Hoopes C, Hays SR, Golden JA. Gastro-oesophageal reflux and aspiration in patients with advanced lung disease. Thorax. 2009; 64:167. [PubMed: 19176842]
31. Sweet MP, Herbella FA, Leard L, et al. The prevalence of distal and proximal gastroesophageal reflux in patients awaiting lung transplantation. Ann Surg. 2006; 244:491. [PubMed: 16998357]
32. Farrell S, McMaster C, Gibson D, Shields MD, McCallion WA. Pepsin in bronchoalveolar lavage fluid: a specific and sensitive method of diagnosing gastro-oesophageal reflux-related pulmonary aspiration. J Pediatr Surg. 2006; 41:289. [PubMed: 16481237]
33. Au J, Hawkins T, Venables C, et al. Upper gastrointestinal dysmotility in heart-lung transplant recipients. Ann Thorac Surg. 1993; 55:94. [PubMed: 8417718]
34. Bodet-Milin C, Querellou S, Oudoux A, et al. Delayed gastric emptying scintigraphy in cystic fibrosis patients before and after lung transplantation. J Heart Lung Transplant. 2006; 25:1077. Epub 2006 Jul 10. [PubMed: 16962469]
35. Berkowitz N, Schulman LL, McGregor C, Markowitz D. Gastroparesis after lung transplantation. Potential role in postoperative respiratory complications. Chest. 1995; 108:1602. [PubMed: 7497768]
36. Tamhankar AP, Peters JH, Portale G, et al. Omeprazole does not reduce gastroesophageal reflux: new insights using multichannel intraluminal impedance technology. J Gastrointest Surg. 2004; 8:890. discussion 897–8. [PubMed: 15531244]
37. Blondeau K, Mertens V, Vanaudenaerde BA, et al. Gastro-oesophageal reflux and gastric aspiration in lung transplant patients with or without chronic rejection. Eur Respir J. 2008; 31:707. [PubMed: 18057058]
38. Hartwig MG, Appel JZ, Davis RD. Antireflux surgery in the setting of lung transplantation: strategies for treating gastroesophageal reflux disease in a high-risk population. Thorac Surg Clin. 2005; 15:417. [PubMed: 16104132]
39. Lau CL, Palmer SM, Howell DN, et al. Laparoscopic antireflux surgery in the lung transplant population. Surg Endosc. 2002; 16:1674. [PubMed: 12140642]
40. Gasper WJ, Sweet MP, Hoopes C, et al. Antireflux surgery for patients with end-stage lung disease before and after lung transplantation. Surg Endosc. 2008; 22:495. [PubMed: 17704875]
41. Linden PA, Gilbert RJ, Yeap BY, et al. Laparoscopic fundoplication in patients with end-stage lung disease awaiting transplantation. J Thorac Cardiovasc Surg. 2006; 131:438. [PubMed: 16434276]
42. O'Halloran EK, Reynolds JD, Lau CL, et al. Laparoscopic Nissen fundoplication for treating reflux in lung transplant recipients. J Gastrointest Surg. 2004; 8:132. [PubMed: 14746846]
43. Palmer SM, Miralles AP, Howell DN, Brazer SR, Tapson VF, Davis RD. Gastroesophageal reflux as a reversible cause of allograft dysfunction after lung transplantation. Chest. 2000; 118:1214. [PubMed: 11035701]
44. Davis RD Jr, Lau CL, Eubanks S, et al. Improved lung allograft function after fundoplication in patients with gastroesophageal reflux disease undergoing lung transplantation. J Thorac Cardiovasc Surg. 2003; 125:533. [PubMed: 12658195]
45. Cantu E 3rd, Appel JZ 3rd, Hartwig MG, et al. J Maxwell Chamberlain Memorial Paper. Early fundoplication prevents chronic allograft dysfunction in patients with gastroesophageal reflux disease. Ann Thorac Surg. 2004; 78:1142. [PubMed: 15464462]
46. D'Ovidio F, Singer LG, Hadjiliadis D, et al. Prevalence of gastroesophageal reflux in end-stage lung disease candidates for lung transplant. Ann Thorac Surg. 2005; 80:1254. [PubMed: 16181849]

47. Benden C, Aurora P, Curry J, Whitmore P, Priestley L, Elliott MJ. High prevalence of gastroesophageal reflux disease in lung transplant recipients. Pediatr Pulmonol. 2005; 40:68. [PubMed: 15880421]
48. Irwin RS, French CL, Curley FJ, Zawacki JK, Bennett FM. Chronic cough due to gastroesophageal reflux. Clinical, diagnostic, and pathogenetic aspects. Chest. 1993; 104:1511. [PubMed: 8222816]
49. Oelschlager BK, Chang L, Pope CE 2nd, Pellegrini CA. Typical GERD symptoms and esophageal pH monitoring are not enough to diagnose pharyngeal reflux. J Surg Res. 2005; 128:55. [PubMed: 16115493]
50. Koufman JA. The otolaryngologic manifestations of gastroesophageal reflux disease (GERD): a clinical investigation of 225 patients using ambulatory 24-hour pH monitoring and an experimental investigation of the role of acid and pepsin in the development of laryngeal injury. Laryngoscope. 1991; 101 Suppl. 53(4 Pt 2):1. [PubMed: 1895864]
51. Oelschlager BK, Pellegrini CA. Minimally invasive surgery for gastroesophageal reflux disease. J Laparoendosc Adv Surg Tech A. 2001; 11:341. [PubMed: 11814124]
52. Oelschlager BK, Eubanks TR, Oleynikov D, Pope C, Pellegrini CA. Symptomatic and physiologic outcomes after operative treatment for extraesophageal reflux. Surg Endosc. 2002; 16:1032. [PubMed: 11984664]
53. Pellegrini CA, DeMeester TR, Johnson LF, Skinner DB. Gastroesophageal reflux and pulmonary aspiration: incidence, functional abnormality, and results of surgical therapy. Surgery. 1979; 86:110. [PubMed: 36677]
54. Eubanks TR, Omelanczuk P, Hillel A, Maronian N, Pope CE, Pellegrini CA. Pharyngeal pH measurements in patients with respiratory symptoms before and during proton pump inhibitor therapy. Am J Surg. 2001; 181:466. [PubMed: 11448445]
55. Vaezi MF, Schroeder PL, Richter JE. Reproducibility of proximal probe pH parameters in 24-hour ambulatory esophageal pH monitoring. Am J Gastroenterol. 1997; 92:825. [PubMed: 9149194]
56. Bredenoord AJ, Tutuian R, Smout AJ, Castell DO. Technology review: esophageal impedance monitoring. Am J Gastroenterol. 2007; 102:187. [PubMed: 17100961]
57. Dransfield MT, Garver RI, Weill D. Standardized guidelines for surveillance bronchoscopy reduce complications in lung transplant recipients. J Heart Lung Transplant. 2004; 23:110. [PubMed: 14734135]
58. Valentine VG, Taylor DE, Dhillon GS, et al. Success of lung transplantation without surveillance bronchoscopy. J Heart Lung Transplant. 2002; 21:319. [PubMed: 11897519]
59. Tiroke AH, Bewig B, Haverich A. Bronchoalveolar lavage in lung transplantation. State of the art. Clin Transplant. 1999; 13:131. [PubMed: 10202611]
60. McWilliams TJ, Williams TJ, Whitford HM, Snell GI. Surveillance bronchoscopy in lung transplant recipients: risk versus benefit. J Heart Lung Transplant. 2008; 27:1203. [PubMed: 18971092]
61. Ward C, Forrest IA, Brownlee IA, et al. Pepsin like activity in bronchoalveolar lavage fluid is suggestive of gastric aspiration in lung allografts. Thorax. 2005; 60:872. [PubMed: 16055614]
62. Stovold R, Forrest IA, Corris PA, et al. Pepsin, a biomarker of gastric aspiration in lung allografts: a putative association with rejection. Am J Respir Crit Care Med. 2007; 175:1298. [PubMed: 17413126]
63. Sidorenko GI, Zborovskiĭ EI, Levina DI. Surface-active properties of the exhaled air condensate (a new method of studying lung function. Ter Arkh. 1980; 52:65. [PubMed: 6892965]
64. Hunt J. Exhaled breath condensate: an overview. Immunol Allergy Clin North Am. 2007; 27:587. [PubMed: 17996577]
65. Walsh BK, Mackey DJ, Pajewski T, Yu Y, Gaston BM, Hunt JF. Exhaled-breath condensate pH can be safely and continuously monitored in mechanically ventilated patients. Respir Care. 2006; 51:1125. [PubMed: 17005057]
66. Muller WG, Morini F, Eaton S, Peters M, Jaffe A. Safety and feasibility of exhaled breath condensate collection in ventilated infants and children. Eur Respir J. 2006; 28:479. [PubMed: 16641124]
67. Hunt J. Exhaled breath condensate: an evolving tool for noninvasive evaluation of lung disease. J Allergy Clin Immunol. 2002; 110:28. [PubMed: 12110814]

68. Horváth I, Hunt J, Barnes PJ, et al. Exhaled breath condensate: methodological recommendations and unresolved questions. Eur Respir J. 2005; 26:523. [PubMed: 16135737]

69. Ono E, Mita H, Taniguchi M, et al. Comparison of cysteinyl leukotriene concentrations between exhaled breath condensate and bronchoalveolar lavage fluid. Clin Exp Allergy. 2008; 38:1866. [PubMed: 19037963]

70. Jackson AS, Sandrini A, Campbell C, Chow S, Thomas PS, Yates DH. Comparison of biomarkers in exhaled breath condensate and bronchoalveolar lavage. Am J Respir Crit Care Med. 2007; 175:222. [PubMed: 17110649]

71. Dupont LJ, Dewandeleer Y, Vanaudenaerde BM, Van Raemdonck DE, Verleden GM. The pH of exhaled breath condensate of patients with allograft rejection after lung transplantation. Am J Transplant. 2006; 6:1486. [PubMed: 16686775]

72. Hunt J, Yu Y, Burns J, et al. Identification of acid reflux cough using serial assays of exhaled breath condensate pH. Cough. 2006; 2:3. [PubMed: 16608520]

73. Kostikas K, Papatheodorou G, Ganas K, Psathakis K, Panagou P, Loukides S. pH in expired breath condensate of patients with inflammatory airway diseases. Am J Respir Crit Care Med. 2002; 10:1364. [PubMed: 12016097]

74. Krishnan A, Chow S, Thomas P, Malouf M, Glanville A, Yates D. Exhaled breath condensate pepsin: a new noninvasive marker of GERD after lung transplantation. J Heart Lung Transplant. 2007; 26 Suppl. 1(2):S139.